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Intra-guild predation and cannibalism between *Harmonia axyridis* and *Adalia bipunctata* adults and larvae: laboratory experiments

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Abstract

Direct interaction between adults and larvae of the ladybird *Harmonia axyridis* (Pallas) (exotic) and *Adalia bipunctata* (L.) (native) Coleoptera Coccinellidae, species was studied in the laboratory. Cannibalism (CANN) and intra-guild predation (IGP) were evaluated using simple bioassays to predict possible impact of exotic on native Coccinellidae. Bioassays were conducted using fourth instar larvae and adult female ladybirds that were individually confined in Petri dishes at 25 °C with 10 second instar larvae of the selected target species. The experiments included three treatments: i) only 10 coccinellid larvae, ii) 10 coccinellid larvae and 5 aphids and iii) 10 coccinellid larvae and 40 aphids. The aphid species used as prey was *Myzus persicae* (Sulzer). No significant differences between IGP and CANN of *H. axyridis* were detected, both for adults and fourth instar larvae. CANN of *A. bipunctata* was always significantly higher in comparison with IGP. In particular for the larvae and adults of *A. bipunctata* the consumption of interspecific larvae was very low. In some cases the females did not even show any predation of *H. axyridis* larvae. IGP of *H. axyridis* was always higher than CANN of *A. bipunctata*. *H. axyridis* showed a negative impact on *A. bipunctata* by direct means such as IGP, contrary to the findings of our previous experiments performed using eggs as the target. For *H. axyridis* in all bioassays on CANN and IGP against larvae, an inverse correlation was detected between the number of larvae consumed *vs* aphid density. For *A. bipunctata* an inverse correlation was detected only in the case of adult CANN. An asymmetric IGP between the two ladybird species, with a potential advantage for the exotic *H. axyridis* was shown. A preliminary risk assessment using these simple bioassays, rather than more complicated experiments, should always be performed before the introduction of a generalists predator as a biocontrol agent in a new agroecosystem.

Key words: Coccinellidae, harlequin ladybird, multicoloured Asian ladybird, *Harmonia axyridis*, two spotted ladybird, *Adalia bipunctata*, *Myzus persicae*, intra-guild predation, cannibalism.

Introduction

Biocontrol using ladybirds was often successful in crop protection (Hodek and Honek, 1996; Ferran *et al.*, 1996). Even if considered beneficial, ladybird inundative releases may however produce negative ecological side effects, due to the polyphagous habits of the coccinellid and the spatial and temporal co-occurrence of the same predatory guild. Some reviews have addressed the issue of non-target impacts of biocontrol (Pimentel *et al.*, 1984; Howarth, 1991; Samways, 1994; Simberloff and Stiling, 1996; Rosenheim *et al.*, 1995; Wajnberg *et al.*, 2001). Only recently, the potential risks of release of exotic natural enemies have received attention outside the biological control world, and many countries now apply risk assessment procedures before a natural enemy can be imported or released (van Lenteren *et al.*, 2003). Negative impacts of introduced exotics include competitive suppression or displacement of native natural enemies and suppression or extinction of non-target prey species, some of which may be beneficial (Elliott *et al.*, 1996). Several studies have sought to understand the ecological impact of intra-guild predation (IGP) between the harlequin ladybird or multicoloured Asian ladybird *Harmonia axyridis* (Pallas) (East Palearctic origin), and other indigenous species either from West Palearctic or from Nearctic regions. Recently Koch (2003) reviewed the non-target effects of the establishment of *H. axyridis* in North America, together with other biological traits of this ladybird.

A methodology for risk assessment has been developed within an EU-financed project (ERBIC = Evaluating Environmental Risks of Biological Control Introductions into Europe), as a basis for regulation of import and release of exotic natural enemies used in inundative forms of biological control. Risk indices of commercially available inundative and classical biological control are also reported and the highest indices were found for polyphagous predators, including the coccinellids, *H. axyridis* and *Hippodamia convergens* Guérin-Menéville, and some species of generalist parasitoids (van Lenteren *et al.*, 2003).

H. axyridis was introduced in France (Ferran *et al.*, 1996). References about permanent establishment in Europe are reported for Germany (Bathon, 2003), Belgium (Adriaens *et al.*, 2003) and recently for Switzerland (Klausnitzer, 2004) and UK (Majerus and Roy, 2005). Introductions were conducted in Greece (Katsoyannos *et al.*, 1997), in Egypt (El-Arnaulty *et al.*, 2000), but it is unclear if the establishment took place in either country. In South America, *H. axyridis* was recorded in Brazil (De Almeida and Da Silva, 2002) and released against aphids in peach orchards in Argentina(Garcia *et al.*, 1999), where it was later also found in urban areas (Saini, 2004). In the United States (LaMana and Miller, 1996; Brown and Miller, 1998; Colunga-Garcia and Gage, 1998; Cottrell, 2004) and Canada (Coderre *et al.*, 1995), *H. axyridis* is certainly established and considered invasive (Hahn and Kovach, 2004; Cottrell, 2005). However, Lucas *et al.* (2002), demonstrated that the three Coccinellidae species occurred at different heights and neither *Coleomegilla maculata* (DeGeer) nor *Coccinella septempunctata* (L.) modified their vertical distribution on apple trees over a 24 hour period in response to the presence of *H. axyridis*. No evidence of damages due to the invasion of the exotic ladybird *H. axyridis*, in fruit orchards was demonstrated by Koch *et al*. (2004) that found direct attack only on ripe raspberries in Minnesota. Laboratory IGP experiments were performed recently by Snyder *et al*. (2004) in microcosms. In such conditions the impact of two exotic predators (*H. axyridis* and *C. septempunctata*) on native Coccinellidae was evaluated and suggested that interactions among larvae could be one mechanism contributing to species replacements in USA.

In northern Italy *H. axyridis* was released in greenhouses in the 1990's. The conditions for permanent establishment are present (Bazzocchi *et al*., 2004) but, as far as we know, in the field this species has yet to be found. Since 2000-2001 the releases of this beneficial insects are supposed to have been stopped in Italy as a consequence of the lack of risk assessment procedures concerning introduction of exotic arthropods for biocontrol.

Ecological effects of *H. axyridis* in northern Italy, including interspecific competition with predatory guild, have not been assessed due to the possible invasiveness of this species when released into the field. Competition between the East Palearctic coccinellid *H. axyridis* and the two spotted ladybird *Adalia bipunctata* (L.) (West Palearctic and Nearctic origin), was therefore investigated. Our aim was to further examine the IGP and CANN of *H. axyridis* and *A. bipunctata* in the laboratory using larvae as targets, to complete the experiments in which eggs where used under both no-choice (Burgio *et al*., 2002) and choice conditions (Santi *et al*., 2003).

The general goal of these studies is to develop rapid and reliable methods of assessment of potential risks of importing and releasing exotic coccinellids and to provide a practical pre-evaluation criterion on potential environmental effects before introduction. We selected *A. bipunctata*, as the native species, because it is abundant in northern Italy agroecosystems (Boriani *et al*., 1998; Burgio *et al*., 1999; Burgio *et al*., 2004). Moreover the two-spotted ladybird shares similar habitats as *H. axyridis* (orchard trees, shrubs and hedgerows) (Hodek and Honek, 1996).

Materials and methods

Insect rearing

Aphids and coccinellids used in experiments were reared as reported in Burgio *et al*., 2002 and in Santi *et al*., 2003. Larvae of all the coccinellid species were fed with *Ephestia kuehniella* (Keller) frozen eggs and adults were fed with *Myzus persicae* (Sulzer) reared on green pea 'Primizia' (*Pisum sativum* L.). Adult coccinellids were maintained in transparent methyl methacrylate cages (40x30x45 cm) and larvae in plastic cylinders (Kartell©) (18 cm in diameter and 18 cm in height) with a ventilation hole on the top lid. Both adults and larvae were kept at 27 ± 2 °C, 60-80% RH and 16:8 LL:DD.

Experiments of intra-guild predation and cannibalism

Laboratory experiments were conducted with IGP and CANN of larvae by adult females and fourth instar larvae of *H. axyridis* and *A. bipunctata*. The studies included: a) CANN in the exotic ($CANN_{ex}$) and in the native species ($CANN_{nat}$); b) IGP of *H. axyridis vs A. bipunctata*; c) IGP of *A. bipunctata vs H. axyridis*.

Preliminary trials using the methyl methacrylate rearing cages with green-pea plants to assess IGP were undertaken. In such a big cage the event frequency was difficult to quantify because of the low number of interactions. Recently, Hoogendoorn and Heimpel, 2004, studying the interactions between *H. axyridis* and *C. maculata* in a field-cage experiment on maize plants, reported very scarce encounters. These results may be due to the short duration of the experiment and to the difference in the distribution of ladybird larvae on the plant, i.e. *H. axyridis* on the upper and *C. maculata* on the lower parts.

Because of this and because of similar findings by Agarwala and Dixon (1992) and Yasuda *et al*. (2004), all our bioassays were conducted in small arenas (Petri dishes). Experiment conditions were: incubator at 25 ± 2 °C, RH = 70 ± 10%, LL:DD 24:0, prey and predators in glass Petri dishes 12 cm in diameter. Pre-test conditions were: predators were fed for 24 hours with aphids and starved for the next 24 hours (Agarwala and Dixon, 1992). Then individuals were placed in Petri dishes with 10 coccinellid second instar larvae of the selected target species toghther with 5 or 40 aphids. After 1 hour observation the remaining number of coccinellid larvae and live aphids were counted. Each bioassay was replicated 30 times. The lab-trials included three treatments: i) with only larvae of coccinellids; ii) with larvae of coccinellids and 5 aphids and iii) with larvae of coccinellids and 40 aphids.

The experiments using females and fourth instar larvae were combined to compare: i) IGP and CANN of *H. axyridis* (IGP_{ex} *vs* $CANN_{ex}$); ii) CANN of *A. bipunctata* and IGP of *H. axyridis* ($CANN_{nat}$ *vs* IGP_{ex}); iii) IGP of *A. bipunctata* and CANN of *A. bipunctata* (IGP_{nat} *vs* $CANN_{nat}$) (table 1).

Data were analysed by non-parametric analysis of variance (Kruskal-Wallis test). A non parametric approach was used because of heteroscedasticity and departures from normality (Zar, 1984). The relationship between larvae consumption and aphid density was analysed by Spearman rank correlation.

Results and discussion

No significant differences between IGP and CANN of *H. axyridis* were detected, both for adults and fourth instar larvae (Kruskal-Wallis test, $P > 0.05$) (figures 1 and 2) with the exception of the experiment performed using larvae with 40 aphids. IGP of the exotic coccinellid species against native species was always higher than CANN of the indigenous *A. bipunctata* (Kruskal Wallis

Table 1. Summary of the correlation analysis of larvae consumption by adults and 4th instar larvae *vs* aphid density.

Predatory species	Target larvae	Behaviour	R	P
H. axyridis adults	*H. axyridis*	*H. axyridis* CANN	−0.80	<0.001
A. bipunctata adults	*A. bipunctata*	*A. bipunctata* CANN	−0.25	0.02
H. axyridis adults	*A. bipunctata*	*H. axyridis* IGP	−0.63	<0.001
A. bipunctata adults	*H. axyridis*	*A. bipunctata* IGP	−0.14	0.22
H. axyridis larvae	*H. axyridis*	*H. axyridis* CANN	−0.45	<0.001
A. bipunctata larvae	*A. bipunctata*	*A. bipunctata* CANN	−0.18	0.12
H. axyridis larvae	*A. bipunctata*	*H. axyridis* IGP	−0.29	0.01
A. bipunctata larvae	*H. axyridis*	*A. bipunctata* IGP	−0.18	0.12

test, $P < 0.05$) (figures 3 and 4). CANN of native *A. bipunctata* was significantly higher in comparison with IGP (figures 5 and 6), for both adults and larvae; in particular for *A. bipunctata*, the consumption of exotic larvae was very low and in some cases females did not show any predation of *H. axyridis* larvae. Adults of *A. bipunctata* did prey on *H. axyridis* larvae only in the experiment with 5 and 40 aphids (figure 5).

H. axyridis showed a partial negative effect for the evidence that IGP of exotic was significant higher in comparison with CANN of native. It appears that the exotic *H. axyridis* have a negative impact on indigenous species *A. bipunctata* by direct means such as IGP in laboratory, contrary to the findings of a previous experiment performed using eggs as the target (Burgio *et al*., 2002).

For *H. axyridis*, in the set of experiments on CANN and IGP against larvae, a significant inverse correlation between the number of attacked larvae by adults and larvae *vs* aphid density was detected (table 1). The Spearman correlation coefficients in the experiments with *H. axyridis* ranged between 0.29 and 0.80. This relationship possibly resulted from a decline in the probability of encountering larvae as aphid density increased, as found by Agarwala and Dixon (1992) for *A. bipunctata*. For the indigenous Coccinellid an inverse

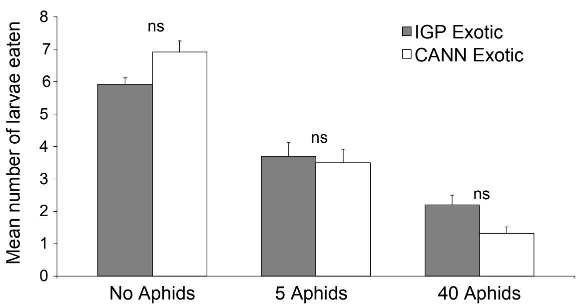

Figure 1. Adults of *H. axyridis*: comparisons between intra-guild predation (IGP_{ex}) and cannibalism ($CANN_{ex}$); target of experiments are *H. axyridis* eggs or *A. bipunctata* eggs.
ns: $P > 0.05$ (Kruskal-Wallis test).

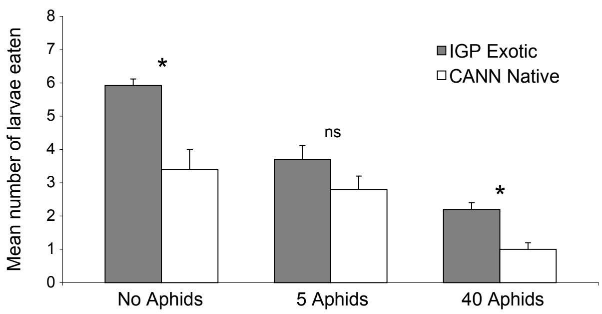

Figure 3. Adults of *A. bipunctata* and *H. axyridis*: comparisons between cannibalism of native ($CANN_{nat}$) and intra-guild predation of exotic (IGP_{ex}); target of experiments are *A. bipunctata* eggs.
*: $P < 0.05$; ns: $P > 0.05$ (Kruskal-Wallis test).

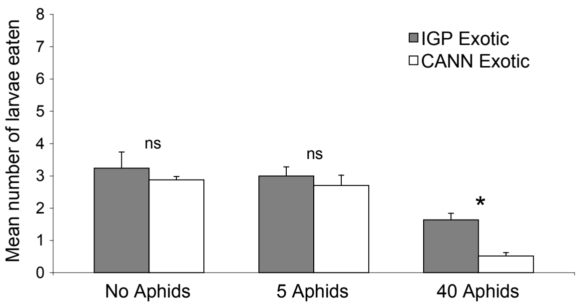

Figure 2. 4th instar larvae of *H. axyridis*: comparisons between intra-guild predation (IGP_{ex}) and cannibalism ($CANN_{ex}$); target of experiments are *H. axyridis* eggs or *A. bipunctata* eggs.
*: $P < 0.05$; ns: $P > 0.05$ (Kruskal-Wallis test).

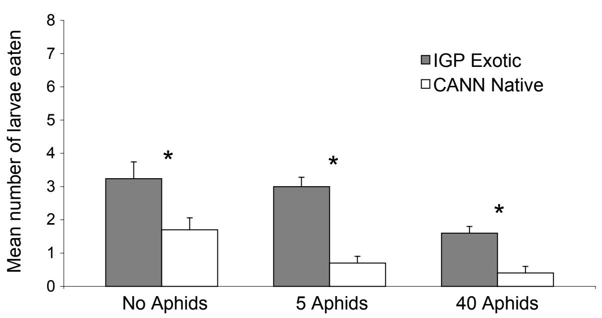

Figure 4. 4th instar larvae of *A. bipunctata* and *H. axyridis*: comparisons between cannibalism of native ($CANN_{nat}$) and intra-guild predation of exotic (IGP_{ex}); target of experiments are *A. bipunctata* eggs.
*: $P < 0.05$ (Kruskal-Wallis test).

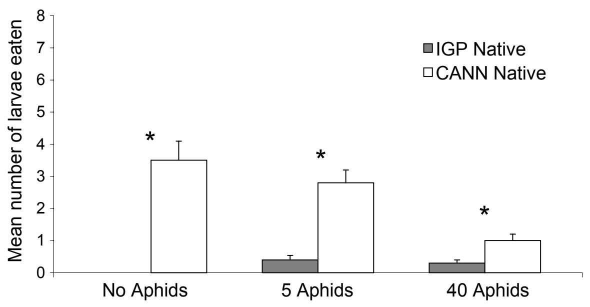

Figure 5. Adults of *A. bipunctata*: comparisons between cannibalism ($CANN_{nat}$) and intra-guild predation (IGP_{nat}); target of experiments are *A. bipunctata* eggs or *H. axyridis* larvae.
*: P < 0.05 (Kruskal-Wallis test).

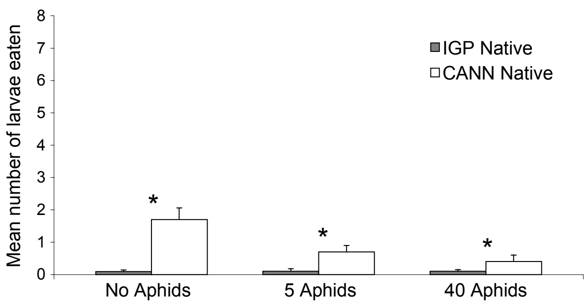

Figure 6. 4^{th} instar larvae of *A. bipunctata*: comparisons between cannibalism ($CANN_{nat}$) and intra-guil predation (IGP_{nat}); target of experiments are *A. bipunctata* larvae or *H. axyridis* larvae.
*: P < 0.05 (Kruskal-Wallis test).

correlation was detected only in the case of adult CANN (table 1); the correlation of CANN of larvae was not significant (R = 0.18, P = 0.12) probably due to the low level of consumption. In the case of IGP, the larvae consumption in *A. bipunctata* was not related to the aphid density, probably for very low levels of IGP of larvae showed by this species. In general the correlation coefficients in the set of experiments was in some cases low and this was probably due to the variability of the larvae consumption rate, as demonstrated in an experiment using eggs as target (Burgio *et al*., 2002).

Our experiments seem to demonstrate that larvae of Coccinellids are in general a IGP prey for adults and larvae of the same family. In *A. bipunctata* IGP of larvae was very low, and in three experiments of six the females of this species did not show any predation events on *H. axyridis* larvae (figures 3 and 4).

Obrycki *et al*. (1998) demonstrated that interspecific interactions, favouring *C. septempunctata* over *C. maculata*, occurred at a low prey density (one aphid per day); no interspecific interactions were observed between larvae of these two species at high prey density (>20 aphids per predator per day). Results of Agarwala and Dixon (1992) indicated that CANN occurs mainly when aphid prey is scarce. This is adaptive in that it improves the chances of survival, and coccinellid, to varying degrees, are defended against interspecific predation. It is confirmed by laboratory bioassays that some species of aphidophagous ladybirds at early stages and without prey prefer CANN over IGP and this phenomenon it is due to repellents (Omkar *et al*., 2004). In *A. bipunctata* larvae and adults the IGP was very low (figures 5 and 6). The females of this species did not show strong predation events on *H. axyridis* larvae.

Probably, in our experiments the reluctance of adults and larvae of *A. bipunctata* to prey on *H. axyridis* second instar larvae can depends on chemical repellence. At any rate the evidence that *H. axyridis* larvae are strongly protected against IGP, can be one of the reasons for the high colonisation potential of this species, but this hypothesis should to be confirmed by specific experiments. Predation experiments showing larval interactions between the Asian ladybird and other Coccinellidae species in which the exotic prevail are reported by Cottrell and Yeargan (1998), Yasuda *et al*. (2004) and Snyder *et al*. (2004). It is clear that IGP is not the only way by which an exotic natural enemy can compete with a native species and other factors are involved, including polyphagy, predatory potential, resource competition and other biological traits. Coccinellids exhibit many interactions in agricultural landscape related to spatial patterns of habitat use (Kieckhefer *et al*., 1992). The seasonal timing of occurrence and reproduction in various habitats (Coderre *et al*., 1995) and the extent of overlap in the distributions of the coccinellid and aphid species on a particular plant species (Coderre and Tourneur, 1986) are factors to be taken into account. These factors determine the predatory potential of a coccinellid in an agroecosystem and for these reasons it is difficult to predict the effect of an introduced species on biological control and on populations of native coccinellid species.

In conclusion, while IGP of exotic against the eggs of native species was never higher than CANN of eggs of native species (Burgio *et al*., 2002), when we considered the larvae as target, in many cases IGP of exotic was higher in comparison with CANN of native. As for *A. bipunctata* adults and larvae, the consumption of exotic (interspecific) larvae was very low and in some cases females did not show any predation of larvae. Our data seem to demonstrate, in the case of larvae predation, an asymmetric IGP to the potential advantage for the exotic *H. axyridis*. Our bioassays on IGP and CANN in a small arena, drastically simplifies the field situation. IGP lab experiments between *H. axyridis* eggs and larvae and the pentatomid *Podisus maculiventris* (Say) showed asymmetric interactions in favour of the pentatomid when the extra-guild prey was low and the test was carried out in small arenas (De Clercq *et al*., 2003). In case of preliminary experiments using caged green-pea, the coccinellids showed no evidence of IGP on eggs (Burgio *et al*., 2002) and larvae (preliminary experiments, see materials and methods) when plants and aphids were present. In a laboratory microcosms containing pea plants the interactions between larvae of *H. axyridis* and *C. transvenoguttata* the Asian ladybird survived multiple encounters with the native species, whereas the native rarely survived a single encounter with *H. axyridis* (Snyder *et al*., 2004). Also Hoogendoorn and Heimpel (2004) in big cages found an asymmetric IGP between the Asian ladybird and the nearctic *C. maculata* in favour of the exotic. They conclude "no increase in the larval mortality of *C. maculata* in presence of *H. axyridis* larvae, nor did we find negative effects on *C. maculata* larval weight gain and food intake in our experiment, suggesting that larval interactions will not negatively affect *C. maculata* populations". For these reasons we chose "extreme" conditions i.e. small arenas to obtain sufficient data in a short time. CANN is also important to evaluate the potential impact of the exotic species *vs* indigenous the fact to conduct bioassays in semi-field or in bigger arenas such as microcosms with plants may predict asymmetric competition but could be difficult to direct examine the IGP and CANN events. We assume that if in small arenas, as we conduct our bioassays, the IGP in comparison with CANN is relevant, similar effects could be predicted also in the field and so is one of the mechanism that could be important to species replacements. Biological traits of the *H. axyridis*, compared to other Italian native species of Coccinellidae, do not seem to be factors that may contribute to the invasiveness of this ladybird (Lanzoni *et al*., 2004). Vice versa, in Japan the two spotted ladybird may compete with Asian ladybird and studies were carried out to study more about their life history in a new establishment area (Sakuratani *et al*., 2000; Sato and Dixon, 2004). Agarwala *et al*. (2003) demonstrated that in open field other interactions such as semiochemicals could be important in predator avoidance. Regarding morphological traits and particularly the body weight, IGP experiments between of *H. axyridis* and *Coccinella undecimpunctata* L. demonstrate that the mobility and body weight of the larvae were not the only important parameters involved in the symmetry of IGP (Felix and Soares, 2004). Sato *et al.* (2005) observed differences in larvae behaviour and Cottrell and Shapiro-Ilan, 2003 reported on susceptibility to fungi infection. In agroecosystems other biotic factors must be taken into account such us predators and parasitoids together with the pesticide impact that could be a further selective mechanism for species replacement. As recently reported by many authors, publishing papers on IGP studies on Asian ladybird (Koch, 2003; Cottrell, 2004; Sato and Dixon, 2004), the predictions of adverse impacts were difficult to assess. *H. axyridis* was first recorded in USA in 1916 (Gordon, 1985) but movement across North America was not reported until the 1990's. Considering the difficulties to avoid accidental introductions and the fact that in several cases commercial insectaries still advertise the sale of exotic beneficials, it is suggested the adoption of a precaution principle before intentional arthropod introductions into new ecosystems (van Lenteren *et al*., 2003).

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